first region, and not exposing a second region of said surface to light;

covalently coupling a first nucleotide to said nucleic acids on said part of said substrate exposed to light, said first nucleotide covalently coupled to said photoremovable group;

exposing a part of said first region of said substrate to light, and not exposing another part of said first region of said substrate to light to remove said photoremovable groups;

covalently coupling a second nucleotide to said part of said first region exposed to light; and

repeating said steps of exposing said substrate to light and covalently coupling nucleotides until said more than 500 nucleotides are formed on said surface.--

REMARKS

I. General

Claims 105 and 107-115 are amended. Claims 106 and 116 have been cancelled, without prejudice. Claims 117-120 have been added for the Examiner's consideration.

The Examiner requests insertion of a reference to the color drawings in the specification. Applicants have added the suggested paragraph to the first page of the application.

The Examiner requires the substitution of a new title that reflects the claims of the current divisional application. Applicants have amended the title to reflect the current claims.

II. Rejections Under 35 USC 112

The Examiner suggests clarification of the phrase "Apparatus" in the claims, and suggests that the dependent claims begin with the term "The." Applicants have amended the claims to be clearer in this regard. The Examiner also suggests that the term "containing" could have ambiguous interpretations in the present context. Applicants have amended the term to recite

"comprising." In addition, the claims now clearly recite that the predefined regions are on "the surface" of the substrate and the oligonucleotides are covalently coupled to the surface. Accordingly, the rejections are overcome.

The Examiner asserts that it is unclear if the "oligonucleotides" in the claims are referring to the individual molecules or the individual sequences, which would often include many molecules. Regarding claim 105, the claim now clearly recites "different" in reference to "oligonucleotide sequences." Applicants clarified claim 110 similarly. Accordingly, it is also clear that the "different" refers to the difference between the sequences of the oligonucleotides, and not the individual molecules. Further, the relationship between "different oligonucleotides" and "predefined regions" is now clear. For the record, Applicants note that the phrase "predefined region" is used to denote that area on the nucleotide where identical sequences are located. However, the claims do not preclude predefined regions having oligonucleotides with the same sequences (e.g., for quality control purposes). Finally, claims 114 and 115 now depend from claim 110, as the Examiner suggests.

The Examiner asserts that the relationship between the "apparatus" and the "substrate" is unclear. By referring now only to the substrate, the rejection is overcome.

The Examiner asserts that the definition of "substantially pure" in claims 109 and 114 is unclear. The claims now refer to a purity (i.e., identical sequences) of greater than 50%. See, e.g., page 29, line 29 for support. Accordingly, by virtue of this alternative approach, it is believed the rejection should be overcome.

Cancellation of claim 116 renders the remaining rejection moot.

III. Rejections Under 35 USC 102

The Examiner notes that the application names joint inventors. Applicants confirm that the claims were commonly owned at the time the inventions covered therein were made.

A. Hames & Higgins / Bio-Rad

Claims 105-107, 109, and 114 are rejected under 35 USC 102(b) as anticipated by the Bio-Rad Catalogue M 1987. Claims 105-106, 109, and 114 are rejected under 35 USC 102 as unpatentable over Hames & Higgins. The Examiner asserts that Bio-Rad shows 3 mm diameter circles of DNA or RNA, while Hames & Higgins shows 4 mm blots of DNA on a substrate. The Examiner equates 3 mm dots with 7,000 square microns.

The rejections are traversed. Most importantly, Applicants claim a substrate wherein the area occupied by the nucleic acids occupy less than 0.01 cm^2 in claim 105, down to 10,000 square microns in claim 107. In the case of Bio-Rad, 3 mm dots are in fact greater than 7,000,000 square microns (not 7,000 square microns). The conversion is outlined below:

$$\pi\left(\frac{3\text{ mm}}{2}\right)^2\left(\frac{1\text{ meter}}{1{,}000\text{ mm}}\right)^2\left(\frac{1\text{ micron}}{1\text{x}10^{-6}\text{ meter}}\right)^2 = 7{,}068{,}583\text{ microns}^2$$

As can be clearly seen from the above conversion, Bio-Rad and Hames & Higgins fall far short of teaching or enabling the present invention. The dimensions of the substrate regions in both references are one to 4 orders of magnitude larger than those claimed herein. Moreover, the references fail to suggest any method for modifying the techniques used therein to achieve the dimensions recited in the present claims. Accordingly, the rejection should be withdrawn.

These references in fact demonstrate the importance of the present invention. Using the Bio-Rad technique, for example, a vacuum source is used to draw various materials through a nitrocellulose substrate. Bio-Rad asserts that it is possible to make 96 different probes on a substrate, each occupying an area

of about 3 mm. Bio-Rad refers to the device as a "microfiltration apparatus," clearly suggesting that 3 mm diameter circles are small scale synthesis sites.

Bio-Rad implies to one of skill in the art that their resolution capability is such that extremely small probe sites are prepared. Bio-Rad contains no suggestion that it would be desirable to make the probe sites smaller, and completely fails to suggest how the device could/would be modified to make the dramatic reductions in size that would lead to the claimed invention.

Bio-Rad includes a near-to-scale photocopy of the 3 mm dots in their advertising material. To appreciate Applicants' invention, one can envision an area of about 1/10 the area shown on that page (in the Bio-Rad reference) to understand the size of the region recited in claim 105. This region would amount to little more than a "speck" on the page. Claim 107 recites an area of about 1/1000'th of the size of the Bio-Rad dot, an area that would likely not be visible to the average eye, or would be virtually indistinguishable from irregularities on a piece of paper.

Clearly, the present claims recite features not foreseen or remotely suggested by Bio-Rad or Hames & Higgins. Accordingly, reconsideration of the rejection is requested.

B. Singer et al. and Schwartz

Claims 105-114 are rejected under 35 USC 102(e) as unpatentable over Singer et al. Claims 105-109 and 114-116 are rejected as unpatentable over Schwartz. By virtue of the amendments to the claims to more clearly recite the invention under 35 USC 112, it is believed that the rejections over Singer et al. and Schwartz have also been rendered largely moot.

Singer et al. discuss a technique in which cells are treated and attached to, e.g., glass coverslips. An example at col. 7 provides for the attachment of skeletal myoblasts of chicken embryos. Thereafter, the coverslip with the cells

thereon is exposed to a labelled receptor. Schwartz, relating to a volume exclusion agent for in-situ hybridization, is largely based on the disclosure of Singer et al. and, accordingly, the disclosure of Singer et al. will be addressed below.

Singer et al. and Schwartz are fundamentally different than the inventions recited herein. According to Singer et al. a plurality of identical cells with RNA or DNA fragments therein of unknown sequence are "plated" on a substrate in a random fashion. Thereafter, labeled, nick-translated receptor DNA fragments are exposed to the cells. These fragments are also of a random sequence by virtue of nick translation. The probes then hybridize to complementary fragments in the cells. Singer et al. use radiographic measurements and others to detect the presence of binding in the cells.

The Singer et al. substrates do not have several recited features of the presently claimed substrates. Importantly, the present invention provides for an ordered set of oligonucleotides of "known" sequence at "known" (or "predefined") locations. By contrast, the random fragments of the cells/receptors in Singer et al. do not provide different, known oligonucleotides at known locations of a substrate. Instead, the cell/receptor fragments of Singer et al. are a largely unknown collection of molecules spread in a generally random fashion over the surface of the substrate, such that the location and identity of no particular oligonucleotide on the surface could be determined with any ease.

A substrate with an array of oligonucleotides with known sequence at known locations of a substrate is useful in many applications for which the Singer et al. substrate would be largely worthless. For example, in hybridization studies it now becomes possible to determine the sequence of an oligonucleotide on a surface that hybridizes to another oligonucleotide, simply through knowledge of its location. Accordingly, one may use the claimed substrate to sequence a particular oligonucleotide receptor. This is one example of an application of the claimed

substrates that Singer et al.'s substrate does not and could not provide.

Moreover, the references fail to show or suggest the covalent coupling of known oligonucleotide sequences to known locations of a substrate in the manner claimed. Although there is little disclosure regarding the manner in which the cells are placed on the substrate, it appears that the cells were mechanically placed on the cell, and then heated for a prolonged period (col.7, line 57 et seq.). There is no suggestion to form an array of nucleic acids covalently coupled to a substrate in the predefined areas claimed.

Accordingly, reconsideration of the rejection is requested.

C. Hames & Higgins in view of Koester et al. and Guire

Claims 115 and 116 are rejected under 35 USC 103 as being unpatentable over Hames & Higgins in view of Koester et al. and Guire. Claim 116 has been cancelled. Claim 115 is now dependent upon claim 110, additionally reciting linker molecules between the substrate and the oligonucleotides.

As amended, claim 115 includes the limitations relating to the molecular diversity in claim 110, which has not been rejected over Hames & Higgins. Koester et al. and Guire do not provide any disclosure regarding the synthesis of diversity at this scale. Accordingly, reconsideration of the rejection in view of the amendments to the claims is requested.

CONCLUSION

For the reasons set forth above, Applicants assert that the claims are in condition for allowance, and early allowance thereof is requested.

If the Examiner believes a telephonic or in person interview would facilitate review of the application, the Examiner is invited to contact the undersigned attorney by telephone at (415) 326-2400.

Respectfully submitted,
TOWNSEND and TOWNSEND KHOURIE and CREW

Date: 11/1/93

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